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## REMARKS

Claims 72-95 have been canceled without prejudice or disclaimer. Claims 96-120 have been added and therefore are pending in the present application. Claims 96-120 are supported throughout the specification. The amount of the cereal recited in claim 96 is supported by Tables 1 and 3 at pages 36 and 40 of the specification.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

### I. The Rejection of Claims 85-87 under 35 U.S.C. 112

Claims 85-87 are rejected under 35 U.S.C. 112 as being indefinite. Specifically, the Office objected to the terms "a sequence" and "an amino acid sequence".

Claims 85-87 have been rewritten as claims 110-112, respectively, to address this rejection. Applicants therefore submit that this rejection has been overcome.

### II. The Rejection of Claims 72-90 under 35 U.S.C. 103

The Office maintained the rejection of claims 72-90 under 35 U.S.C. 103 as being unpatentable over Lischnig et al. (Biotechnology Letters, Vol. 15, No. 4, pp. 411-414 (1993)) or Gomes et al. (Appl. Microbiol. Biotechnol., Vol. 39, pp. 700-707 (1993)) or Alam et al. (Enzyme Microb. Technol., Vol. 16, pp. 298-302 (1994)) and Haarasilta et al. (U.S. Patent No. 5,314,692) and Hazlewood et al. (WO 93/25693). This rejection is respectfully traversed.

As mentioned in the prior response, none of the cited references teach or suggest the use of thermostable xylanases in animal feed compositions, as claimed herein.

Moreover, none of the cited references teach or suggest that there would be any advantage to using a thermostable xylanase over a thermolabile xylanase in animal feed. As explained in the prior responses, animal feeds comprising a thermostable xylanase of Family 11 according to the present invention have significantly improved feed utilization over animal feeds comprising other xylanases.

In response to Applicants' showing of surprising and unexpected results, the Office stated:

> While the findings of Dr. Pettersson are acceptable to the Examiner, the labeling of said results for all feeds and in all animals is unacceptable.... Examiner notes that the publication of Dr. Pettersson et al. is limited to studying the effect of *T. lanuginosus* xylanase on a single feed componenet, wheat, and is restricted to

> chicken fed said wheat and in comparison to only two other xylanases. The study is also limited to the use of a single *T. lanuginosus* enzyme and its comparison to only two other xylanases. However, the claims are drawn to animal feed comprising component, not limited to wheat, as well as not limited to feeding only chicken. On similar lines, the enzyme claimed to be comprised in the claimed animal feed is not limited to a single enzyme i.e., xylanase comprising SEQ ID NO: 2 but those that have an amino acid sequence 95% identical to SEQ ID NO: 2 and those xylanases encoded by polynucelotides which can hybridize to nucleotides 31-705 of SEQ ID NO: 1 under a specific set of stringent conditions. Therefore, while the evidence provided by Dr. Pettersson can be considered as unexpected for SEQ ID NO: 2, such consideration cannot be given to all variants and mutants of SEQ ID NO: 2 as claimed because such unexpected results have not been demonstrated for those variants and mutants or with feeds comprising more wheat as a feed component and fed to a representative number of different animals. Therefore, while applicants' arguments are persuasive for an animal feed comprising wheat as a main component, comprising SEQ ID NO: 2 as the xylanase enzyme, wherein said feed is made for feeding chicks or poultry, their arguments in support for animal feed comprising any component, comprising a genus of xylanases including variants and mutants of SEQ ID NO: 2, for feeding any or all animals, as claimed, are not persuasive.

This is respectfully traversed.

Applicants submit that Applicants' showing of surprising and unexpected results is commensurate with the scope of the claims.

The claimed invention is drawn to animal feed compositions comprising a xylanase of Family 11 glycosyl hydrolase having a pH-optimum in the range of 4.5-7.5 and a residual xylanase activity after incubation for 60 minutes at pH 6.0 of one or more of: more than 96% residual activity when measured at 60°C; more than 83% residual activity when measured at 65°C; more than 20% residual activity when measured at 70°C; and more than 10% residual activity when measured at 75°C, wherein the xylanase comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 2 and improves the growth rate and/or feed coversion ratio of a chick or poultry.

Thus, the claimed invention does not comprise all xylanases having such an amino acid sequence. The claims also specify that the xylanase should have other properties, *e.g.*, that it be a Family 11 glycosyl hydrolase and have high thermostability. Applicants determined that xylanases having these properties have significantly improved feed utilization over animal feeds comprising other xylanases, including a commercially-available xylanase product, namely BIO-FEED PLUS, a xylanase preparation from *Humicola insolens*. Based on Applicants' data, one

skilled in the art would expect that the results apply to xylanases having these properties and are not limited to the xylanase having the sequence of SEQ ID NO: 2.

With respect to the other components contained in the animal feeds, Applicants have demonstrated that the xylanases of the present invention have significantly improved feed utilization over animal feeds comprising other xylanases in a composition comprising wheat. Both wheat and rye cereals have a high content and high solubility of arabinoxylans (see, Pettersson and Åman, 1987, *Acta Agric. Scand.* 37:20-26 (a copy of which is attached hereto)). Thus, persons skilled in the art would expect that Applicants' surprising and unexpected results also would be obtained by animal feed compositions comprising wheat and/or rye.

Applicants also respectfully submit that requiring applicants to limit the claims as suggested by the Examiner would be contrary to public policy and would not adequately protect the inventors. Based on Applicants' teachings of the present application, one would attempt to circumvent the literal scope of Applicants' patent rights by feeding Applicants' animal feed compositions to different animals. Furthermore, one would add a different cereal than wheat and still obtain Applicants' improved feed utilization. Finally, one would attempt to use a xylanase having the same properties as the xylanase of SEQ ID NO: 2 and obtain improved feed utilization.

For the foregoing reasons and the reasons set forth in the prior responses, Applicants submit that the claims overcome this rejection under 35 U.S.C. 103. Applicants respectfully request reconsideration and withdrawal of the rejection.

## III. The Rejection of Claims 72-90 under the Doctrine of Obviousness-Type Double Patenting

The Office maintained the rejection of claims 72-90 under the doctrine of obviousness-type double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 6,245,546.

As mentioned in the prior response, Applicants will submit a terminal disclaimer upon an indication of allowable subject matter.

## IV. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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# The Variation in Chemical Composition of Triticales Grown in Sweden

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Pettersson, D. & Åman, P. (Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, S-75007 Uppsala, Sweden). The variation in chemical composition of triticales grown in Sweden. Received March 3, 1986. Acta Agric Scand 37: 20–26, 1987.

The gross composition of 80 samples of winter-triticale, 5 of winter-rye and 10 of winter-wheat, grown in the south of Sweden was investigated. On average, the triticale samples contained 66.5% starch, 13.3% total fibre, 11.7% crude protein, 4.6% free sugars, 2.2% crude fat and 1.8% ash. The highest coefficient of variation was obtained for the free sugars and the lowest for starch. Compared to the reference rye and wheat samples, the triticale samples contained higher amounts of crude protien. The contents of soluble, insoluble and total fibres were highest in the rye, lowest in the wheat and generally intermediate in the triticale samples. However, the highest amounts of insoluble pentosans were observed in the triticale samples. The amounts of water soluble pentosans were lowest in wheat, highest in rye and intermediate in triticale, and were directly proportional to extract viscosity. *Key words: triticale, starch, fibre, crude protein, pentosans, viscosity.*

## INTRODUCTION

By crossing wheat with rye it is possible to produce a new species, triticale, which may combine the high yield of wheat with the favourable protein composition of rye. Although a triticale was described already in 1876, it was not until the possibility of chromosomal doubling through colchicin treatment was discovered (Blakeslee, 1937) that it was possible to produce fertile, high yielding and enduring species. Today, triticale yields are comparable to wheat (Larter, 1974; Shebeski, 1974) and problems such as kernel shrivelling, sprouting and poor winter endurance which originally were associated with this grain have been reduced (Wolski & Tymieniecka, 1975; Zillinsky, 1975; Ericksson et al., 1978). Unfortunately the protein content has also diminished.

In Sweden an interest has developed for the use of modern high-yielding triticales as an alternative crop grain. For this purpose the development of new suitable varieties of winter-hardy triticale was initiated. The investigation presented in this paper was carried out by using modern analytical methods to determine the chemical composition and variation of triticales. Since there has been no such complete investigation in Scandinavia, this work was undertaken to provide the basic information necessary for the evaluation of nutrient quality of modern triticales grown in Sweden.

## MATERIALS AND METHODS

### *Material*

Samples of winter triticale ($n=80$) were obtained from the plant breeding companies Svalöf AB and Weibullsholm AB located in the south of Sweden. The samples represent 27 cultivars or lines, two localities (56°N) and two growing seasons (1983 and 1984). The Swedish triticale samples included a few old lines from the mid-fifties while the majority was of modern offspring, and genetic variations of the same line was were also represent-

ed. Several samples of the Polish cultivar Lasko and the Russian cultivars AD 201 and 206, all grown in Sweden, were also included. A reference material consisting of rye (cv Kungs II), wheat (cv Holme) and three triticales; Lasko, WW 31433 and Sv 8008 was grown near Landskronn both years. This reference material has been used in extensive physiological experiments, chemical investigations and feeding trials by several research groups in Sweden.

### *Methods*

Prior to analysis representative samples (100 g) of the grains (89.7–92.0% dry matter) were ground in a Tecator cyclone sample mill to pass a 0.5 mm screen.

The dry matter content was determined by oven-drying at 105°C for 5 h and all analyses, which were carried out in duplicate, are reported on a dry matter basis. Free glucose, fructose, sucrose and fructans were extracted with 0.05 M Na-acetate buffer (pH 5.0) at 65°C and determined enzymatically (Larsson & Bengtsson, 1983). Crude protein and ash were determined according to standard methods (AOAC, 1980). Crude fat was extracted with diethyl ether in a Tecator Soxtec System HT after acid hydrolysis (Anonymous, 1971). Starch was determined by an enzymatic method (Åman & Hesselman, 1984). Total fibre was calculated by subtracting the contents of free sugars, starch, crude protein, crude fat and ash from the dry matter of the sample (Åman & Hesselman, 1984).

Grain samples (500 mg) were pre-extracted (2×30 min) with 2×15 ml 80% ethanol in an ultrasonic water bath. The residues obtained on centrifugation (1500 g, 10 min) were further extracted (2 h) with water (15 ml) in the ultrasonic water bath maintained at a temperature less than 28°C. The supernatant was collected after centrifugation (1800 g, 15 min) and viscosity was calculated relative to the extraction media (water) after measurements on an Ostwald viscometer kept at 30°C. Part of the supernatant (1 ml) was blown to dryness, hydrolysed with TFA and the formed sugar residues converted to alditol acetates and analysed by GLC (Albersheim et al., 1967). Water-soluble pentosans were calculated as the sum of the xylose and arabinose residues.

The contents of soluble and insoluble non-starch polysaccharide residues and Klason lignin were determined according to Theander & Åman (1979) and total dietary fibre was calculated as the sum of non-starch polysaccharide residues and Klason lignin.

The statistical analyses were performed by using the Statistical Analysis System (SAS Institute Inc., 1982).

## RESULTS

The variation in gross chemical composition of the 80 samples of triticale grains is presented in Table 1. Starch was the major constituent followed by total fibre and crude protein. On average these three components together constituted 91.5% of the dry matter. The average content of free sugars (glucose, fructose, sucrose and fructans) was 4.6%, with sucrose as dominating constituent. The average contents of both crude fat and ash were approximately 2% The coefficients of variation were lowest for starch followed by total fibre and crude protein and highest for the free sugars.

First order regression analysis revealed statistically significant relationships of crude protein (coefficient of regression −0.31; $p<0.001$) and total fibre (coefficient of regression, −0.48; $p<0.001$) on starch. However, the coefficients of determination were low ($R^2=0.15$ and 0.19 respectively). First order regression analysis of total fibre on crude protein revealed a statistically significant negative relationship (coefficient of regression −0.58; $p<0.001$), with a low coefficient of determination ($R^2=0.18$).

Gross chemical composition of the reference material showed an average content of free

sugars of 6.1% in the rye cultivar, 3.1% in the wheat cultivar and 4.0, 4.3 and 5.4% respectively in the triticale samples (Table 2). The high content of fructans in the rye cultivar was notable. The starch content was highest in the wheat cultivar followed by the triticale cultivar Lasko. Crude protein content was highest in the three triticales, while the rye cultivar had the highest content of total fibre.

The content and composition of soluble and insoluble dietary fibres in the reference material are presented in Table 3. The content of soluble dietary fibres was highest in the rye cultivar (4.0%) and lowest in the wheat and triticale cultivar Lasko (both 1.9%). The average content of solube dietary fibres in the 10 triticales, selected to contain a large variation in total fibre, was 2.2%. Arabinose, xylose and glucose residues were major constituents of soluble dietary fibres in all samples.

The content of insoluble dietary fibres was also highest in the rye cultivar (12.5%) and lowest in the wheat and Lasko cultivars (both 8.5%). The average content of insoluble dietary fibres in the 10 triticales was 11.7% and arabinose, xylose and glucose residues were also the main constituents of this fraction in all samples. Therefore, total dietary

Table 1. *Variation in chemical composition (% of dry matter) of grain of triticale lines (n=80) grown in Sweden*

| Chemical constituent | Mean value | Range of values | Coefficient of variation (%) |
|---|---|---|---|
| Glucose | 0.5 | 0.1–1.3 | 58.2 |
| Fructose | 0.1 | 0.1–0.2 | 29.1 |
| Sucrose | 3.2 | 2.0–4.9 | 26.7 |
| Fructans | 0.8 | 0.2–1.5 | 32.1 |
| Starch | 66.5 | 61.9–69.5 | 2.5 |
| Crude protein (Nx6.25) | 11.7 | 9.4–16.5 | 11.2 |
| Crude fat | 2.2 | 1.0–2.9 | 17.5 |
| Ash | 1.8 | 1.1–3.0 | 18.7 |
| Total fibre[a] | 13.3 | 9.7–18.0 | 13.6 |

[a] Determined by difference.

Table 2. *Chemical composition (% of dry matter) of the reference material (means±standard error)*

| | Rye | Wheat | Triticale | | |
|---|---|---|---|---|---|
| | Kangs II $n=2$ | Holme $n=6$ | Lasko $n=4$ | Sv8008 $n=3$ | WW31433 $n=2$ |
| Glucose | 0.4 | 0.2±0.04 | 0.4±0.1 | 0.4±0.1 | 0.6 |
| Fructose | 0.1 | 0.1±0.01 | 0.1±0.02 | 0.1±0.01 | 0.1 |
| Sucrose | 2.9 | 1.8±0.1 | 2.7±0.1 | 3.1±0.3 | 4.0 |
| Fructans | 2.7 | 1.0±0.1 | 0.8±0.03 | 0.7±0.3 | 0.7 |
| Starch | 65.6 | 70.3±0.4 | 68.1±0.5 | 64.9±1.0 | 65.0 |
| Crude protein (Nx6.25) | 9.5 | 10.7±0.4 | 12.1±0.2 | 12.8±0.5 | 11.4 |
| Crude fat | 2.4 | 2.7±0.01 | 2.3±0.02 | 2.2±0.04 | 2.6 |
| Ash | 1.9 | 1.6±0.04 | 1.6±0.1 | 2.1±0.3 | 2.0 |
| Total fibre[a] | 14.5 | 11.7±0.3 | 11.9±0.6 | 13.7±0.6 | 13.8 |

[a] Determined by difference.

fibres were highest in the rye (16.5%) and lowest in the wheat and Lasko cultivars (10.4 and 10.5% respectively). The average content of total dietary fibre in the 10 triticales was 13.9%.

Water extraction below 28°C gave extracts in which arabinose and xylose residues were the dominating carbohydrates (compare Fig. 1). When calculated as percent of original grain dry matter the arabinose residues constituted 0.63% (range 0.52–0.71%) and the xylose residues 0.86% (range 0.71–0.99%) in 5 rye samples. In 54 triticale samples the arabinose residues constituted 0.36% (range 0.26–0.48%) and xylose residues 0.35% (range 0.26–0.49%), while the arabinose residues in 10 wheat samples constituted 0.24% (range 0.19–0.30%) and the xylose residues 0.22% (range 0.17–0.27%). The average ratio of arabinose to xylose residues in these water-soluble pentosans were 1:1.4, 1:1.0 and 1:0.9 in rye, triticale and wheat respectively.

When the content of water-soluble pentosans was plotted against relative viscosity, segregated populations were obtained for the rye, triticale and wheat samples respectively (Fig. 1). The highest relative viscosity and content of soluble pentosans was found in the rye samples, the lowest in the wheat samples while the triticale samples were intermediate.

## DISCUSSION

The triticale material analysed in the present investigation represents mainly modern Swedish high-yielding breeding-lines, but also a few interesting Polish, Russian and old Swedish cultivars and lines. High coefficients of variation were obtained for the free sugars in triticale in accordance with findings concerning barley and oats (Åman et al., 1985; Åman, 1987). On the other hand, the variation in starch content of triticale was low

Table 3. *Content of soluble, insoluble and total dietary fibres and of non-starch polysaccharide residues and Klason lignin in the soluble and insoluble fractions in the reference material (% of dry matter) and in 10 varieties of triticale (% of dry matter, mean ± standard error)*

| | Rye | Wheat | Triticale | | | |
|---|---|---|---|---|---|---|
| | Kungs II | Holme | Lasko | Sv 8008 | WW31433 | Average of 10 triticales |
| *Soluble* | | | | | | |
| Arabinose | 1.03 | 0.41 | 0.49 | 0.68 | 0.56 | 0.54±0.03 |
| Xylose | 1.62 | 0.65 | 0.54 | 0.78 | 0.61 | 0.61±0.03 |
| Mannose | 0.18 | 0.07 | 0.21 | 0.16 | 0.21 | 0.19±0.01 |
| Galactose | 0.17 | 0.21 | 0.21 | 0.23 | 0.22 | 0.22±0.01 |
| Glucose | 1.01 | 0.56 | 0.43 | 0.63 | 0.53 | 0.63±0.09 |
| Total | 4.0 | 1.9 | 1.9 | 2.5 | 2.1 | 2.2±0.1 |
| *Insoluble* | | | | | | |
| Arabinose | 2.22 | 1.86 | 2.08 | 2.49 | 2.67 | 2.59±0.08 |
| Xylose | 2.67 | 2.04 | 2.19 | 2.96 | 3.08 | 2.96±0.10 |
| Mannose | 0.43 | 0.23 | 0.35 | 0.29 | 0.39 | 0.35±0.01 |
| Galactose | 0.33 | 0.17 | 0.17 | 0.27 | 0.24 | 0.26±0.01 |
| Glucose | 3.83 | 2.67 | 2.68 | 2.92 | 3.14 | 3.15±0.08 |
| Klason lignin | 3.0 | 1.5 | 1.0 | 2.8 | 1.5 | 2.4±0.33 |
| Total | 12.5 | 8.5 | 8.5 | 11.7 | 11.0 | 11.7±0.5 |
| Total dietary fibres | 16.5 | 10.4 | 10.5 | 14.2 | 13.1 | 13.9±0.5 |

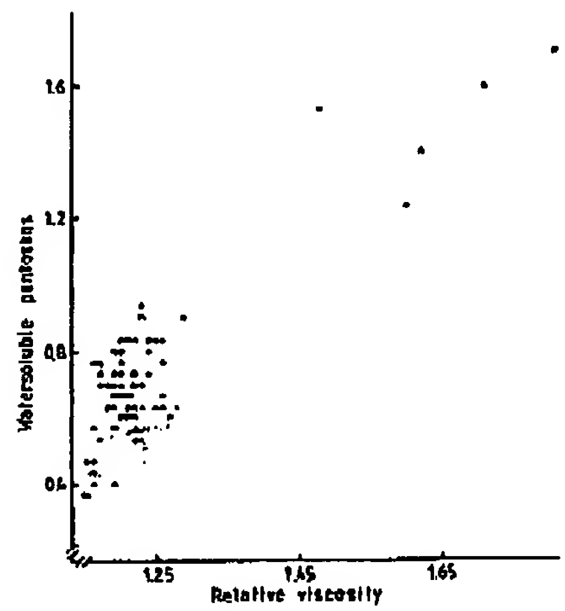


*Fig. 1.* Plot of water-soluble pentosans, quantified as arabinose and xylose residues soluble below 28°C (% of dry matter) versus relative viscosity. ○ = wheat samples; ● = triticale samples; □ = rye samples.

compared to barley and oats. This may be explained by the lower variation in growing conditions since the triticale material represents only two localities in the south of Sweden and two years. Triticale may have a disturbed starch synthesis which can lead to a low starch content and also a shrivelling of the grain (Hill et al., 1973; Dedio et al., 1975). Compared to barley, where the starch content may vary from 21 to 64% (Åman & Newman, 1986), the small variation obtained in the present investigation does not indicate any major disturbance in the investigated triticales.

Three low-yielding Russian samples had unusually high protein contents (AD 201, 15.5%; AD 206, 15.1 and 16.5% respectively) and when these samples were excluded the range of protein content for the triticales was significantly reduced (9.4–13.4%). In the literature a wide variation in protein content is presented (Villegas et al., 1970:Zillinsky & Borlaug, 1971; Zillinsky, 1975) and a disturbed starch synthesis is reported to increase the protein content (Bushuk, 1980). Compared to barley (Åman et al., 1985) and oats (Åman, 1987) the variation in crude fat and ash contents were large.

The negative coefficients of regression revealed that in triticale, on average, the total fibre and crude protein contents increased by 0.48 and 0.31 %-units respectively when the starch content decreased by 1 %-unit. These results are similar to results on barley (Åman et al., 1985) while a significantly higher figure for total fibre and lower figure for crude protein was obtained for oats (Åman, 1987). As in oats, but not in barley, a negative relationship between total fibre and crude protein was obtained.

In the reference material, the wheat sample was characterized by the lowest contents of free sugars, ash and total fibre and the highest contents of starch and crude fat, while the rye sample revealed the highest contents of fructans and total fibre and the lowest crude protein content. The triticale samples contained intermediate amounts of free sugars and total fibre and the highest content of crude protein. The gross chemical composition of Lasko revealed a close resemblance to the wheat sample.

The average total fibre content of triticales calculated by difference (13.3%) did not differ significantly from the analysed dietary fibre content (13.9%), a relationship which

also has been demonstrated for other cereals (Åman & Hesselman, 1984; Åman et al., 1985; Åman, 1987). The contents of soluble, insoluble and total dietary fibres were highest in the rye sample and lowest in the wheat sample. The triticale samples generally contained intermediate amounts of the fibre components, but Lasko showed a close resemblance to the wheat sample and Sv 8008 contained high amounts of soluble arabinose and xylose residues. It is also notable that the contents of insoluble arabinose and xylose residues were high in all triticale samples except Lasko.

Rye is known to have a high content of pentosans which form viscous solutions and are known to be responsible for the poor performance of broiler chickens fed rye-based diets (Antoniou et al., 1981; Antoniou & Marquardt, 1981). The rye in the present investigation contained high amounts of soluble pentosans, resulting in viscous solutions, while the pentosan content in wheat was low. The triticale samples contained intermediate amounts of these pentosans and the extracts had intermediate viscosities. It is notable that the triticale samples were closer related to the wheat samples although segregated populations were obtained.

The results presented in this paper demonstrate a restricted variation in starch content of modern triticale samples while considerable variations were noted for other chemical components. In many respects the triticale samples showed a composition intermediate between that of wheat and of rye, although the crude protein content and the amount of insoluble pentosans were high.

## ACKNOWLEDGEMENTS

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